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#### **Original Articles**

## Intercalibration of benthic foraminiferal and macrofaunal biotic indices: An example from the Norwegian Skagerrak coast (NE North Sea)



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#### ABSTRACT

The present study illustrates how benthic macrofauna indices can be adapted to foraminifera through intercalibration of data from common sites. As an example of how benthic foraminifera can fit into governmental monitoring programs, we focus on Norwegian conditions by proposing a new foraminifera-based multimetric index,  $NQI_f$ . The index is an adaptation of the Norwegian Quality Index (NQI), which is an internationally intercalibrated macrofauna index.

The study is based on published and new data for soft-bottom benthic foraminifera, macro invertebrates, and associated bottom water dissolved oxygen and sediment total organic carbon (TOC). Paired samples of foraminifera and macrofauna were collected at the same stations, at more or less the same time, along the Norwegian Skagerrak coast, NE North Sea. The intercalibration was based on linear regression and the EcoQS class boundary values for the foraminifera indices were derived from boundary values for the macrofauna indices defined by the Norwegian governmental guidelines. The correlations between foraminifera and macrofauna for the multimetric NQI and the diversity indices  $H'log_2$  and  $ES_{100}$  were all acceptable for intercalibration (according to the Water Framework Directive's guidelines) but NQI showed the best correlation. Both foraminifera- and macrofauna-indices showed significant correlations with the bottom water dissolved oxygen concentration, and for some indices, with the TOC content in the sediment. Overall, the foraminifera and macrofauna indices reflected the environmental conditions similarly but at the most oxygen depleted stations only foraminifera were present. Based on the present findings and on previous studies which show a potential of fossil foraminifera to define *in situ* reference conditions, we recommend that foraminifera are accepted as a Biological Quality Element within the WFD.

#### 1. Introduction

The European Water Framework Directive (WFD, 2000) emphasises that the ecological quality status (EcoQS) of transitional and coastal waters shall be evaluated based on Biological Quality Elements (BQEs). Benthic macro invertebrates (from now on termed macrofauna) is one of the selected BQEs used. For each BQE, biotic indices have been developed that can classify transitional and coastal waters into five classes of EcoQS: «high», «good», «moderate», «poor», and «bad». In order to determine whether or not the EcoQS of a water body has been negatively impacted by human activity, information about the reference conditions is needed to calculate the Ecological Quality Ratio (EQR). "Reference conditions are a description of the biological quality elements at high status" (WFD, 2000, p. 39) and EQR quantifies (on a

numerical scale from zero to one) the relation between observed and reference condition values of a BQE. Since defining the reference conditions is a recurring problem (WFD, 2000, p. 41) there is a need for alternative methods.

Like macrofauna, foraminifera (amoeboid protists), are important members of the marine benthic community. Living foraminifera reflect environmental conditions in the bottom water and sediment surface layers (see overview in e.g., Murray, 2006). Hence, they have recently been suggested as a monitoring tool to characterise the EcoQS (Alve et al., 2009). Later investigations from widely different environments in Greece to the Arctic support this view (e.g., Bouchet et al. 2012, 2018a; Dimiza et al., 2016; Dijkstra et al., 2017). Their small (usually < 0.5 mm) shells (tests) preserve well in ageing sediments, making the foraminifera a commonly used tool in paleoecology and, lately, in

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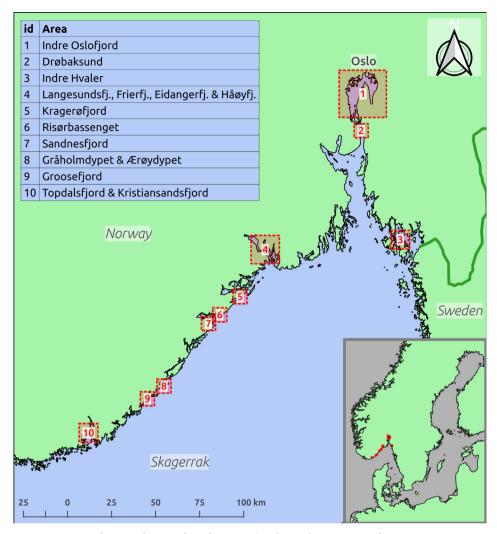


Fig. 1. Study areas along the Norwegian Skagerrak coast, NE North Sea.

reconstruction of past ecological quality status, PaleoEcoQS, and *in situ* reference conditions (e.g., Alve et al., 2009; Dolven et al., 2013; Polovodova Asteman et al., 2015; Romano et al., 2016; Francescangeli et al., 2016). Hence, foraminifera can complement macrofauna-based monitoring and could be included as a governmental assessment tool for EcoQS in soft-bottom habitats. A foraminifera-based classification system intercalibrated with that for macrofauna is then required. Internationally, there are numerous biotic indices in use for both groups of organisms, but the present study focuses on foraminiferal indices equivalent to the macrofauna indices used in the Norwegian classification system.

The usefulness of biodiversity as a measure of ecosystem quality is widely recognized (Laurila-Pant et al., 2015, and references therein) but most countries, including Norway, use multimetric indices that include a sensitivity component in addition to the diversity component (Veileder, 2013). The diversity indices H'log<sub>2</sub> (Shannon and Weaver, 1963) and ES<sub>100</sub> (Hurlbert, 1971) as well as the multimetric Norwegian Quality Index (NQI, Rygg, 2006) are used in the Norwegian classification system. NQI includes a sensitivity component (AMBI) and a diversity factor (lnS/ln(lnN)). A foraminifera equivalent to the macrofauna-based sensitivity index AMBI (Borja et al., 2000), was recently developed based on benthic foraminiferal assemblages from North-East Atlantic and Arctic shelves and fjords (Alve et al., 2016). The Foram-AMBI (AMBI<sub>f</sub>) provides a potential sensitivity component for a multimetric index and opens an opportunity for defining a foraminifera index which can be compared to and intercalibrated with the already

internationally intercalibrated macrofauna counterpart, NQI. Hence, this study proposes  $\mathrm{NQI}_{\underline{f}_{9}}$  a foraminifera-based index similar to the macrofauna-based NQI.

Internationally, vast efforts have been put into intercalibrating EcoQS class boundaries, especially between countries where the same types of water bodies occur (e.g., Borja et al., 2007, 2009; Grémare et al., 2009). The intercalibrations aim to secure a comparable status classification and a valid implementation of the Water Framework Directive throughout e.g., the North-East Atlantic Geographical Intercalibration Group (NEAGIG) which includes the Atlantic coastal areas from northern Norway to Gibraltar. NQI is one among several macrofaunal indices in NEAGIG. NQI has been intercalibrated with indices used in other countries for the water types NEA1/26 (shallow, fully saline) and NEA7 (deep, fully saline) in 2006 (Borja et al., 2007; Carletti and Heiskanen. 2009), in the NEA8/9/10 (Skagerrak and Kattegat) in 2011 (8.10.2013 Official Journal of the European Union L 266/1), and in the NEA1/26 and NEA7 in 2015 (Van Hoey et al., 2018). A comparison of performance along stress gradients of three Scandinavian indices, NQI (Norway), BQI (Sweden) and DKI (Denmark), was made by Josefson et al. (2009). Valid intercalibration procedures are outlined in several documents (e.g. EC, 2011; Van Hoey et al. 2007, 2010, 2015).

As a possible first step to implement foraminifera in official monitoring systems, the present study from Norwegian waters aims to 1) define a multimetric foraminifera-based biotic index,  $NQI_f$ , similar to the macrofauna-based NQI (from now on termed  $NQI_m$ ), 2)

Table 1
Details of study areas, organism groups (F = Foraminifera; M = Macrofauna), sampling equipment (GC = Gravity corer; BC = Box corer; VG = van Veen grab), number of replicates analyzed, and source of data (B-M = Buhl-Mortensen et al., 2009). For location of study areas, see Fig. 1.

Area no.	Study area	Date collected	Station	Latitude	Longitude	Water depth (m)	Org. group	Sampling equip. F/M	No. repl. F/ M	Source
1	Inner Oslofjord	18. Feb. 2009	Fk-41	59.744434	10.550500	122	F	GC	3	New data
	Inner Oslofjord	26. Feb. 2009	Fk-41	59.744350	10.550450	121	M	VG	4	Berge et al. (2011)
	Inner Oslofjord	23. Apr. 2009	Cj-31	59.844734	10.510067	58	F	GC	3	New data
	Inner Oslofjord	10. Mar. 2009	Cj-31	59.844734	10.510067	58	M	VG	4	Berge et al. (2011)
	Inner Oslofjord	23. Apr. 2009	Cp-31	59.835415	10.706800	101	F	GC	3	New data
	Inner Oslofjord	12. Mar. 2009	Cp-31	59.835415	10.706800	101	M	VG	4	Berge et al. (2011)
	Inner Oslofjord	23. Apr. 2009	Ep-41	59.789551	10.718650	152	F	GC	3	New data
	Inner Oslofjord	11. Mar. 2009	Ep-41	59.789551	10.718650	153	M	VG	4	Berge et al. (2011)
	Inner Oslofjord	18. Feb. 2009	Fk-31	59.756935	10.547167	34	F	GC	3	New data
	Inner Oslofjord	26. Feb. 2009	Fk-31	59.755383	10.543834	33	M	VG	4	Berge et al. (2011)
	Inner Oslofjord	17. Feb. 2009	Gl-21	59.715065	10.572166	64	F	GC/VG	3	New data
	Inner Oslofjord	25. Feb. 2009	Gl-21	59.715034	10.572050	64	M	VG	4	Berge et al. (2011)
	Inner Oslofjord	18. Feb. 2009	El-31	59.784115	10.575233	146	F	GC	3	New data
	Inner Oslofjord	09. Mar. 2009	El-31	59.783150	10.574687	146	M	VG	2	Berge et al. (2011)
	Inner Oslofjord	19. Feb. 2009	Bo-21	59.890549	10.665517	54	F	GC	3	New data
	Inner Oslofjord	24. Feb. 2009	Bo-21	59.890701	10.665433	54	M	VG	4	Berge et al. (2011)
	Inner Oslofjord	19. Feb. 2009	Dm-21	59.826351	10.616199	85	F	GC	3	New data
	Inner Oslofjord	10. Mar. 2009	Dm-21	59.828133	10.616800	85	M	VG	4	Berge et al. (2011)
	Inner Oslofjord	19. Feb. 2009	Cl-31	59.844067	10.576333	70	F	GC	3	New data
	Inner Oslofjord	23. Feb. 2009	Cl-31	59.843399	10.578600	73	M	VG	4	Berge et al. (2011)
	Inner Oslofjord	18. Feb. 2009	El-11	59.797585	10.570000	125	F	GC	3	New data
	Inner Oslofjord	09. Mar. 2009	El-11	59.797352	10.568517	124	M	VG	4	Berge et al. (2011)
	Inner Oslofjord	19. Feb. 2009	Cm-41	59.837715	10.622133	35	F	GC	3	New data
	Inner Oslofjord	10. Mar. 2009	Cm-41	59.837101	10.621767	37	M	VG	4	Berge et al. (2011)
	Inner Oslofjord	24. June 2008	RC5	59.882210	10.746994	54	F/M	GC/VG	3/1	Hess et al. (2014)
	Inner Oslofjord	09. June 2010	RC5	59.882210	10.746994	54	F/M	GC/VG	3/1	Hess et al. (2014)
	Inner Oslofjord	24. June 2008	RC9	59.880730	10.746785	49	F/M	GC/VG	3/1	Hess et al. (2014)
	Inner Oslofjord	09. June 2010	RC9	59.880730	10.746785	49	F/M	GC/VG	3/1	Hess et al. (2014)
2	Drøbaksund	17. Feb. 2009	Im-4x	59.645035	10.613633	157	F	GC	3	New data
	Drøbaksund	25. Feb. 2009	Im-41	59.627651	10.624033	201	M	VG	4	Berge et al. (2011)
3	Indre Hvaler	19. Aug. 2008	IH30	59.113050	11.002567	30	F/M	GC/VG	3/4	Bouchet et al. (2012)
	Indre Hvaler	19. Aug. 2008	IH45	59.108350	10.996883	45	F/M	GC/VG	3/4	Bouchet et al. (2012)
	Indre Hvaler	19. Aug. 2008	IH60	59.103350	10.996600	62	F/M	GC/VG	3/4	Bouchet et al. (2012)
4	Langesundsfj.	07. Aug. 2003	105	59.029517	9.739717	107	F/M	GC/VG	2/5	Alve & Husum (2006); B-M
	Håøyfjorden	07. Aug. 2003	102	59.021850	9.799433	200	F/M	GC/VG	2/5	Alve & Husum (2006); B-M
	Outer Eidangerfj.	07. Aug. 2003	106	59.059833	9.712167	98	F/M	GC/VG	3/5	Alve & Husum (2006); B-M
	Outer Eidangerfj.	20. Aug. 2008	106	59.059833	9.712167	103	F/M	GC/VG	3/4	Bouchet et al. (2012)
	Inner Eidangerfj.	07. Aug. 2003	107	59.086233	9.707733	89	F/M	GC/VG	1/5	Alve & Husum (2006); B
										M
	Frierfjorden	21. Aug. 2008	F30	59.099900	9.640733	28	F/M	GC/VG	3/4	Bouchet et al. (2012)
	Frierfjorden	21. Aug. 2008	F50	59.101783	9.630883	52	F/M	GC/VG	3/4	Bouchet et al. (2012)
	Frierfjorden	21. Aug. 2008	F70	59.103017	9.628767	70	F/M	GC/VG	3/4	Bouchet et al. (2012)
5	Kragerøfjorden	08. Aug. 2003	71	58.831317	9.476083	138	F/M	GC/VG	2/5	Alve & Husum (2006); B-M
	Kragerøfjorden	22. Aug. 2008	71	58.831000	9.476167	138	F/M	GC/VG	3/4	Bouchet et al. (2012)
	Kragerøfjorden	22. Aug. 2008	ΚØ	58.825567	9.467217	102	F/M	GC/VG	3/4	Bouchet et al. (2012)
6	Risørbassenget <sup>1</sup>	08. Aug. 2003	52	58.739167	9.253167	179	F/M	GC/VG	1/2	Alve & Husum (2006); B-
	Diograposses	22 412 2000	D60	E0 7/1067	0.211017	60	E /M	CCAIC	2 /4	M Poughot et al. (2012)
	Risørbassenget	22. Aug. 2008	R60	58.741067	9.311017	60	F/M	GC/VG	3/4	Bouchet et al. (2012)
	Risørbassenget	23. Aug. 2008	R80	58.741350	9.309083	80	F/M	GC/VG	3/4	Bouchet et al. (2012)
	Risørbassenget	23. Aug. 2008	R100	58.741033	9.303783	104	F/M	GC/VG	3/4	Bouchet et al. (2012)
7	Sandnesfjorden <sup>1</sup>	09. Aug. 2003	50	58.696167	9.173667	64	F/M	GC/VG	2/5	Alve & Husum (2006); B-M
	Sandnesfjorden	25. Aug. 2008	50	58.696167	9.173667	65	F/M	GC/VG	3/4	Bouchet et al. (2012)
8	Gråholmdypet	10. Aug. 2003	Grå	58.370667	8.720833	193	F/M	GC/VG	2/5	Alve & Husum (2006); B-M
	Ærøydypet <sup>1</sup>	10. Aug. 2003	200	58.405667	8.776667	109	F/M	GC/VG	2/5	Alve & Husum (2006); B-
										M

(continued on next page)

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Table 1 (continued)

Area no.	Study area	Date collected	Station	Latitude	Longitude	Water depth (m)	Org. group	Sampling equip. F/M	No. repl. F/	Source
9	Groosefjorden	12. Aug. 2003	G69	58.320000	8.592167	69	F/M	GC/VG	1/5	Alve & Husum (2006); B-M
	Groosefjorden	25. Aug. 2008	G69	58.320217	8.592700	69	F/M	GC/VG	3/4	Bouchet et al. (2012)
	Groosefjorden	25. Aug. 2008	G50	58.322483	8.592083	54	F/M	GC/VG	3/4	Bouchet et al. (2012)
10	Topdalsfjorden <sup>1</sup>	11. Aug. 2003	6	58.174000	8.062000	74	F/M	BC + GC/VG	2/5	Alve & Husum (2006); B-M
	Topdalsfjorden	26. Aug. 2008	6	58.174000	8.062000	74	F/M	GC/VG	3/4	Bouchet et al. (2012)
	Kristiansandsfj.	26. Aug. 2008	KDR	58.133617	7.974267	23	F/M	VG/VG	3/4	Bouchet et al. (2012)
	Kristiansandsfj.	26. Aug. 2008	KDC	58.136167	7.976000	31	F/M	GC/VG	3/4	Bouchet et al. (2012)

<sup>&</sup>lt;sup>1</sup> Foraminiferal data partly based on 0–1 cm (replicate A).

intercalibrate NQI $_{\rm m}$  and NQI $_{\rm m}$  and establish class boundaries for NQI $_{\rm f}$  harmonised with NQI $_{\rm m}$ , 3) establish class boundaries for H'log $_{\rm 2,f}$  and ES $_{\rm 100,f}$ , based on correlations with macrofauna, and 4) test these indices against stress gradients (bottom-water dissolved [O $_{\rm 2}$ ] and sediment TOC). By intercalibrating NQI $_{\rm f}$  with NQI $_{\rm m}$ , the foraminifera index can be connected to the NEAGIG family of macrofauna indices. Potentially, NQI $_{\rm f}$  can participate in future NEAGIG international intercalibrations.

#### 2. Methods

The material used in the present study is based on published and new data from the Norwegian Skagerrak coast, NE North Sea, where samples of soft-bottom invertebrate macrofauna and benthic for-aminifera were collected at the same stations at more or less the same time (Fig. 1, Table 1). Samples were collected from 15 stations in the Oslofjord and from 24 stations in a total of 13 fjords and silled basins further south. The samples were collected between 2003 and 2010 and some stations were sampled twice during this time period. The stations represented habitats with stable temperatures (mostly 5–6 °C) and salinities (33–34) but with a wide range in bottom water dissolved oxygen concentration. For details of the investigation areas, see Buhl-Mortensen et al. (2009), Berge et al. (2011), Bouchet et al. (2012), Dolven et al. (2013), Hess et al. (2014).

The macrofauna samples were collected using a  $0.1\,\mathrm{m}^2$  van Veen grab. During most sampling events four or five replicate samples per station were collected and analyzed. The samples were washed through a 1 mm mesh sieve and the retained fraction was preserved in buffered 4–6% formaldehyde in seawater. Collected specimens were, as far as possible, identified to species level.

The foraminiferal samples were collected using gravity cores retrieving cores with 67 mm inner diameter in 2003, with 56 mm diameter at stations RC5 and RC9, and with 80 mm diameter using a Gemini corer (modified from Niemistö, 1974) during all other sampling events (Table 1). In most cases, three replicates were collected and analyzed per station. Each core top was sectioned into either 1 cm or 2 cm thick slices depending on the purpose of the original studies. Consequently, for consistency and the wish to include as many samples as possible, data from the surface 0-2 cm sediment are used in the present study. The samples were preserved in rose Bengal-stained 70% ethanol (1 g L<sup>-1</sup>), see discussion in Murray and Bowser (2000), left for at least two weeks, washed through 500 and 63 µm mesh sieves, and (except for the RC-samples) the 63-500 µm fraction was split using a modified Elmgren wet splitter (Elmgren, 1973). One fourth or one eighth of each sample (depending on foraminiferal abundance) was resieved and all live (stained) foraminifera in the 63-125 and 125-500 µm fractions were picked (in exceptional cases just counted) in the wet state (Duffield and Alve, 2014) and, as far as possible, identified to species level and counted. The number of individuals > 500 µm relative to smaller ones was trivial (< 0.1%) so including them would not influence the results. Only species considered fossilisable in sediments

along the Norwegian Skagerrak coast are included in the present data set (see Bouchet et al., 2012).

During the 2003-, 2008- and 2010-cruises, bottom water from just above the sediment–water interface in one or two gravity cores per station was transferred to Winkler bottles immediately after collection, sealed, and kept dark and cold ( $\sim\!7\,^\circ\text{C})$  for subsequent dissolved oxygen analysis.

For the macrofauna samples, the Shannon-Wiener ( $H'log_2$ ) (Shannon and Weaver, 1963) and the Hurlbert ( $ES_{100}$ ) (Hurlbert, 1971) diversity indices, the sensitivity index AMBI (Borja et al., 2000), and the multimetric NQI (Rygg, 2006) were calculated. For the foraminifera, the same two diversity indices ( $H'log_2$  and  $ES_{100}$ ) were calculated as well as AMBI $_f$  (Alve et al., 2016), and the new foraminifera-based version of NQI (NQI $_f$ , see below). Furthermore, following Bouchet et al. (2012), the diversity index exp ( $H'_{bc}$ ) was calculated to assess EcoQS. The index values for each station-time (some stations were sampled twice, Table 1) were based on the average of replicate values from each sampling event.

The Norwegian Quality Index NQI<sub>m</sub> (NQI; Rygg, 2006) is a multimetric macrofauna index composed of the following metrics:

- (i) A sensitivity component AMBI (AZTI Marine Biotic Index)
- (ii) A diversity factor lnS/ln(lnN) (where S is the number of taxa, N is abundance)
- (iii) A correction factor for down-weighting artificially high diversity values of small samples (few individuals; N/N + 5)

The index is an algorithm where equal weight is given to sensitivity (50%) and diversity (50%) and it is formulated as follows:

$$NQI_{m} = 0.5 \left(1 - \frac{AMBI}{7}\right) + 0.5 \left(\frac{\frac{lnS}{\ln(lnN)}}{2.7}\right) \left(\frac{N}{N+5}\right)$$

The following formula is suggested for the foraminifera NQI f:

$$NQI_{f} = 0.5 \left(1 - \frac{AMBI_{f}}{7}\right) + 0.5 \left(\frac{ES100_{f}}{35}\right)$$

Since foraminiferal samples usually contain more than 100 individuals,  $\mathrm{ES}_{100}$  is considered a good choice for the diversity component of  $\mathrm{NQI}_{\mathtt{L}^{\mathrm{f}}}$ . Like in the  $\mathrm{NQI}_{\mathtt{L}^{\mathrm{m}}}$  index, equal weight (50%) is given to the diversity and sensitivity components. In both NQI indices, the observed sensitivity- and diversity values are divided by their highest obtainable values, respectively. Intercalibration between  $\mathrm{NQI}_{\mathtt{f}}$  and  $\mathrm{NQI}_{\mathtt{m}}$  was based on linear regression analyses and class boundary values for  $\mathrm{NQI}_{\mathtt{f}}$  were derived from boundary values for  $\mathrm{NQI}_{\mathtt{m}}$  as defined by the current Norwegian governmental guidelines (Veileder, 2013) using x and y values of the regression line (Excel regression function). Class boundary values for  $\mathrm{H'log}_{2,\mathtt{f}}$  and  $\mathrm{ES}_{100,\mathtt{f}}$  were determined in the same way, i.e. based on corresponding  $\mathrm{H'log}_{2,\mathtt{m}}$  and  $\mathrm{ES}_{100,\mathtt{m}}$  values.

The biotic indices (average of replicates per station) were tested

along environmental stress gradients represented by dissolved bottom water  $[O_2]$  at the time of sampling and associated sediment TOC values, using the Excel correlation function.

#### 3. Results

The dissolved oxygen concentration in the bottom water at the time of sampling represented a gradient from 0.04 to  $4.76 \,\mathrm{mL}\,\mathrm{O}_2\mathrm{L}^{-1}$ . The TOC values were in the range 0.8-9.0% (Appendix Table 1). A total of 178 and 125 samples, including replicates, were analyzed for macrofauna and foraminifera, respectively. The foraminifera showed both their highest and lowest abundances in the most oxygen depleted basins. On the other hand, while the macrofauna also had their lowest abundances in the oxygen depleted basins, their highest abundances occurred in the well oxygenated basins (Appendix Table 1). Foraminifera were present in all collected samples, whereas a few samples were devoid of macrofauna (of which only station Cp31 is listed in Appendix Table 1). Thirteen foraminiferal and 51 macrofaunal samples had < 100 individuals, i.e. too few for calculating ES<sub>100</sub>. The total number of macrofaunal and foraminiferal species recorded across all samples were 356 and 119, respectively. The most abundant foraminiferal species was Stainforthia fusiformis (Williamson) making up 1-100% of the assemblages (Appendix Table 1).

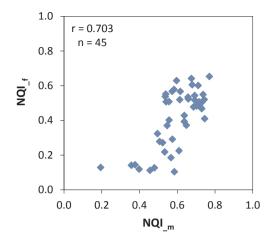
The regression and correlation statistics for comparison of the foraminifera and macrofauna-based biotic indices were all significant (p < 0.001, Table 2, for average of replicates per station, see Appendix Table 1). With Pearson's correlation coefficient  $r \geq 0.5$ , all correlations were adequate for intercalibration (EC, 2011, p. 77) but NQI (Fig. 2) showed a stronger correlation (r = 0.70) than  $H^1 og_2$  (r = 0.58) and  $ES_{100}$  (r = 0.52). The  $H^1 og_2$  values were lower for foraminifera than for macrofauna; 0.1–4.0 and 0.2–5.0, respectively. The same was the case for  $ES_{100}$ , with values 2.1–23.1 for foraminifera and 7.5–38.5 for macrofauna. The calculated inter-calibrated class boundaries and ranges for foraminifera and the associated macrofauna class boundaries and ranges (Veileder, 2013) are shown in Table 3.

The relative abundance of *Stainforthia fusiformis* showed a strong negative correlation (r =  $-0.92,\ p < 0.001$ ) with NQI $_f$  and a somewhat weaker correlation (r =  $-0.71,\ p < 0.001$ ) with bottom water dissolved [O $_2$ ] (Fig. 3). Three of the highest abundances of *S. fusiformis* (86–98%) occurred in two ephemerally hypoxic basins in 2008 (Fig. 3b; stations 6, G69, and G50 in Appendix Table 1). These two basins had bottom water dissolved [O $_2$ ] of 0.75 and 0.50 mLO $_2$ L $^{-1}$  in 2003 whereas the values in 2008 were 2.53 and 2.95 mLO $_2$ L $^{-1}$ , respectively. Similarly, three of the lowest NQI $_f$  values (0.12–0.20) were from the same two basins in 2008 (Fig. 4a), as well as three of the four highest sediment TOC values (6.7–9.0%) in the whole investigation area (Appendix Table 1).

Correlation values for biotic indices vs the dissolved  $[O_2]$  and TOC, respectively, are shown in Table 4 and the correlations between dissolved  $[O_2]$  and  $NQI_f$  and  $NQI_m$ , respectively, are illustrated in Fig. 4. All indices showed significant correlations with the dissolved  $[O_2]$  (p < 0.01) but the correlations were stronger for the foraminifera indices (r > 0.70) than for the macrofauna indices (r < 0.70). All foraminifera indices were significantly negatively correlated with TOC (p < 0.001) and the correlation was strong for AMBI $_f$ ,  $NQI_f$  and

**Table 2** The relationship between macrofauna- (m) and foraminifera- (f) based biotic indices (NQI, H'log<sub>2</sub> and ES<sub>100</sub>). The correlations were all acceptable (r  $\geq$  0.5, EC 2011, p. 77).

	Regression equation	$R^2$	r	p	n
NQI <sub>f</sub> vs NQI <sub>m</sub>	y = 0.9967x - 0.1813 $y = 0.5574x + 0.7401$ $y = 0.3184x + 7.2213$	0.4936	0.703	< 0.0001	45
H'log <sub>2,f</sub> vs H'log <sub>2,m</sub>		0.341	0.584	< 0.0001	46
ES <sub>100,f</sub> vs ES <sub>100,m</sub>		0.2755	0.520	0.00059	40



**Fig. 2.** Correlation between the foraminifera-based NQI $_{\rm f}$  (new) and the macrofauna-based NQI $_{\rm m}$  (Rygg, 2006). Data points represent the average of replicate samples for each of foraminifera and macrofauna assemblages collected at nearly the same time at the same stations along the Norwegian Skagerrak coast

 $H^{\prime}log_{2,f}$  (r > 0.59). Correlations of AMBI $_m$ , NQI $_m$ , and  $H^{\prime}log_{2,m}$  with TOC were significant (p < 0.05) but strong only for NQI $_m$  (r > 0.50). The confidence level was > 95% for all correlations.

#### 4. Discussion

#### 4.1. Foraminifera as a monitoring tool

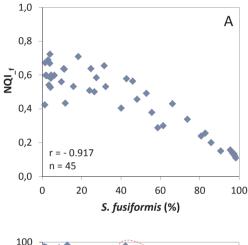
Whereas it is necessary to take a large sediment sample (commonly 0.1 m<sup>2</sup>) to obtain the macrofauna, benthic foraminifera are readily sampled in an 8 cm diameter core, because of their small size and high densities. Time needed for sample processing and taxonomic discrimination is about the same for both groups. For aminifer amay provide information about EcoQS in habitats where the abundance of macrofauna is too low to provide reliable input for the calculation of index values (e.g., Bouchet et al., 2018b). In addition to indices calculated for both groups the present study supports recent findings that, when based on foraminifera, the diversity index  $\exp{(H'_{bc})}$  reliably can assess EcoQS and palaeoEcoQS (Bouchet et al., 2012, 2018a; Dolven et al., 2013; Francescangeli et al., 2016; Dijkstra et al., 2017). Further, because of their short life cycle (months rather than years as for macrofauna) foraminifera are able to respond more quickly to environmental change (Schönfeld et al., 2012). Importantly, foraminiferal shells are preserved as a historical record in the sediment and so can be used to establish past reference conditions for EcoQS (PaleoEcoQS) beyond time intervals represented by instrumental and biological time series (Alve et al., 2009). The latter is particularly relevant for implementation of the WFD, since adequate historical data are non-existent for most investigation areas. There are, however, potential pitfalls associated with retrospective studies including e.g., possible disturbance of the sediments and taphonomic loss or gain of species (discussions in e.g. Martin, 1999; Murray, 2006; Alve et al., 2009). To minimize effects of post-mortem test destructions only species considered fossilisable (Bouchet et al., 2012) were included in the present study.

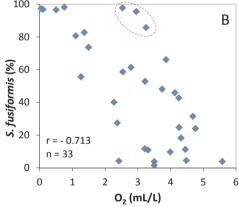
#### 4.2. Methodological considerations

Traditionally the invertebrate macrofauna has been, and still is, the preferred organism group to assess ecological quality in soft-bottom sediments in coastal waters. There are principal differences as well as similarities between macrofauna to macrofauna index intercalibration, and foraminifera to macrofauna index intercalibration. For the latter,

Table 3
Ecological Quality Status (EcoQS) class boundaries for macrofauna (m) (Veileder, 2013, revised 2015) and foraminifera (f) (new data) expressed by three intercalibrated biotic indices, NQI, H'log<sub>2</sub>, ES<sub>100</sub>. The class boundary values for the foraminiferal indices were calculated from boundary values for macrofauna using the trendline equations in Table 2.

EcoQS/Index	Organism group	High	Good	Moderate	Poor	Bad
NQI m	M	1.0-0.72	0.72-0.63	0.63-0.49	0.49-0.31	0.31-0
NQI f	F	1.0-0.54	0.54-0.45	0.45-0.31	0.31-0.13	0.13-0
H'log <sub>2 m</sub>	M	5.7-4.8	4.8-3.0	3.0-1.9	1.9-0.9	0.9-0
H'log <sub>2 f</sub>	F	5.0-3.4	3.4-2.4	2.4-1.8	1.8-1.2	1.2-0
ES <sub>100_m</sub>	M	50-34	34–17	17–10	10-5	5–0
ES <sub>100_f</sub>	F	35–18	18–13	13–11	11–9	9–0





**Fig. 3.** Correlation between *Stainforthia fusiformis* (%) and A) NQI  $_{\rm f}$  and B) the bottom water dissolved oxygen concentration (mL  $O_2L^{-1}$ ). Data points represent average of replicate samples for each of foraminifera and macrofauna assemblages collected at the same stations at nearly the same time along the Norwegian Skagerrak coast. Dashed, red oval object = ephemeral hypoxic basins.

index values are calculated from different data sets (different groups of organisms), whereas in the macrofauna to macrofauna intercalibration, the datasets are identical (index values calculated from exactly the same samples). The similarity is that the data represent a common site and habitat, sampled at the same time, i.e., the foraminifera and the macrofauna indices classify the same target. Furthermore, both groups are composed of benthic and sedentary species, hence their distribution patterns directly depend on the local environmental conditions. Recently, foraminiferal and macrofaunal community compositions in samples collected at the same stations at the same time in SE Norwegian fjords were significantly correlated (cross-taxon congruence) implying that benthic foraminiferal distribution patterns parallel those of benthic macrofauna (Bouchet et al., 2018b). Consequently, there is a conceptual basis for expecting that an intercalibration of indices based on

the two taxonomic groups is justified.

Foraminifera have been shown to be more tolerant of severe hypoxia than other meiofaunal, macrofaunal and megafaunal groups (e.g., Josefson and Widbom, 1988; Gooday et al., 2010). The tolerance is partly due to an ability of some foraminiferal species to perform anaerobic metabolism (e.g., Risgaard-Petersen et al., 2006). In the present study, the difference in tolerance is illustrated by foraminiferal presence in samples devoid of macrofauna. Nevertheless, both foraminifera and macrofauna showed a significant correlation between [O<sub>2</sub>] and the biotic indices (Table 4).

Little information is available concerning comparisons of biotic index values for the two groups based on data from common habitats. However, based on total (live plus dead) foraminiferal assemblages, Wlodarska-Kowalczuk et al. (2013) showed that foraminiferal and macrofaunal diversities (H') in an Arctic glacial fjord were positively correlated. In Italian transitional waters, the assessed EcoQS for foraminifera and macrofauna were similar when based on diversity indices (exp  $(H'_{bc})$  and  $H'log_2$ ,). Despite some discrepancies, diversity-based foraminiferal indices and sensitivity-based macrofauna indices showed similar trends (Bouchet et al., 2018a). Also less directly comparable studies point to similarities (and some differences) between foraminiferal and macrofaunal responses to environmental pressures e.g., sewage outfalls, industrial activity, oil-based drilling mud, and aquaculture (e.g., Schafer et al., 1975; Schafer et al., 1995; Mojtahid et al., 2008; Denoyelle et al., 2010). Concerning EcoQS assessments, similar results were obtained from macrofauna and fossil (sub recent) foraminifera in the Oslofjord using H'log2 and ES100 for both groups and, additionally, exp (H'<sub>bc</sub>) for the foraminifera (Dolven et al., 2013).

#### 4.3. Biotic indices and environmental parameters

All indices showed significant correlations with the environmental parameters (Table 4) indicating that they reliably assessed the environmental quality. Equally good correlations are often seen in other macrofauna-based studies (Muxika et al., 2005; Bouchet and Sauriau, 2008; Borja et al., 2011). It further supports previous works showing that foraminifera-based indices are relevant indicators of environmental conditions (Bouchet et al., 2012, 2018a; Alve et al., 2016). The fact that NQI f and the other indices did not show even better correlations with the [O2] is probably, to some extent, due to a mismatch between the oxygen concentration in the bottom water and that of the sediment pore water (where most foraminifera live) at three stations in two ephemerally hypoxic basins (Figs. 3b and 4a). While the foraminiferal parameters at these stations were more or less the same in 2003 and 2008 (Appendix Table 1), the bottom water [O<sub>2</sub>] in the basins was substantially higher in 2008, probably due to deep water renewal during the winter 2007/2008. This lack of change in foraminiferal parameters indicates that the foraminifera did not, during the months between the renewal and time of collection in August 2008, respond to increased oxygen availability in the bottom water. The explanation is probably related to the high sediment TOC at these three stations relative to the other stations (Appendix Table 1). Oxygen consumption is substantial during degradation of labile organic material and, when

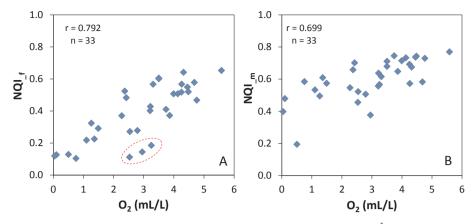


Fig. 4. Correlation between A)  $NQI_f$  and B)  $NQI_m$ , and the bottom water dissolved oxygen concentration (mL  $O_2$  L<sup>-1</sup>). Dashed, red oval object = ephemeral hypoxic basins. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4 Statistics for biotic indices vs bottom water dissolved oxygen concentration (mL  $\rm O_2~L^{-1}$ ) and associated sediment TOC. Biotic index values were based on average of replicates.

$ES_{100\_f}$	$AMBI_{\underline{f}}$	$NQI_{\underline{f}}$	$H\log_{2_f}$	Exp ( $H'_{bc}$ )	S. fusiformis
0.611 0.781 < 0.0001 33	0.565 0.752 < 0.0001 33	0.627 0.792 < 0.0001 33	0.641 0.801 < 0.0001 33	0.605 0.778 < 0.0001 33	0.508 -0.713 < 0.0001 33
0.227 -0.477 < 0.001 45 ES <sub>100_m</sub>	0.430 -0.656 < 0.0001 46 AMBI <sub>_m</sub>	0.366 -0.605 < 0.0001 45 NQI_m	0.353 -0.594 < 0.0001 46 H1og <sub>2_m</sub>	0.218 - 0.467 0.001 46	0.482 0.694 < 0.0001 46
0.274 0.523 0.004 29	0.338 0.581 < 0.001 32	0.489 0.699 < 0.0001	0.359 0.599 0.0002 33		
3					
	0.611 0.781 < 0.0001 33 : 0.227 - 0.477 < 0.001 45 ES <sub>100_m</sub> 0.274 0.523 0.004 29	0.611 0.565 0.781 0.752 < 0.0001 < 0.0001 33 33 : 0.227 0.430 - 0.477 - 0.656 < 0.001 < 0.0001 45 46 ES <sub>100.m</sub> AMBI <sub>.m</sub> 0.274 0.338 0.523 0.581 0.004 < 0.001	0.611 0.565 0.627 0.781 0.752 0.792 < 0.0001 < 0.0001 < 0.0001 33 33 33 0.227 0.430 0.366 - 0.477 - 0.656 - 0.605 < 0.001 < 0.0001 < 0.0001 45 46 45 ES <sub>100,m</sub> AMBI <sub>_m</sub> NQI <sub>_m</sub> 0.274 0.338 0.489 0.523 0.581 0.699 0.004 < 0.0001 < 0.0001	0.611	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$

exceeding supply from the overlying water masses, it causes oxygen depleted sediment pore water (see review in Levin et al., 2009). Hence, the foraminiferal indices in these ephemerally hypoxic basins probably reflected the oxygen conditions in the sediment pore water in 2008 rather than those measured in the bottom water at the time of sampling. Consequently, in ephemerally hypoxic basins, foraminifera may reflect the general environmental conditions in the basin better than an arbitrary oxygen measurement representing a "snap shot" of a time series. This is useful information for interpretations of fossil assemblages.

The present study indicates that sediment TOC is not necessarily an accurate parameter to use in ecological studies, probably because TOC comprises everything from labile, easily degradable to refractory, almost inert organic material (see review in Kristensen, 2000). This implies that low TOC content consisting of labile organic material in one area may cause a higher oxygen consumption than high TOC consisting of refractory organic material in another. Additionally, since only labile material is a good food source for benthic organisms (Jorissen et al., 1995; Middelburg and Levin, 2009), TOC is not necessarily a useful quantitative guide to food supply. These factors may partly explain the moderate instead of high correlations between the biotic indices and TOC in the present study (Table 4).

#### 4.4. Intercalibration

In the present study, the foraminifera-macrofauna correlations of biotic indices were all significant and acceptable for intercalibration (EC, 2011, p. 77) but the multimetric index, NQI, showed a better correlation than the diversity indices (Fig. 2, Table 2). The calculated class boundary values were lower for the foraminifera-based than for the macrofauna-based indices except for the Poor/Bad boundary in the two diversity indices (Table 3). The generally lower boundary values for foraminifera are explained by the overall smaller number of foraminiferal species compared to macrofauna (119 vs 356, respectively). The smaller number of foraminiferal species is also reflected by lower foraminifera vs macrofauna maximum values for H¹log<sub>2</sub> (4.0 vs 5.0) and ES<sub>100</sub> (23.1 vs 38.5, respectively).

The higher foraminiferal class boundary between Poor and Bad in the two diversity indices is explained by the frequent occurrence of Stainforthia fusiformis (Foram-AMBI ecological group V, Alve et al., 2016) in the assemblages at high stress levels (here low bottom water [O<sub>2</sub>], Fig. 3). Stainforthia fusiformis is a typical opportunist (Alve, 2003) strongly dominating in severely oxygen depleted basins along the Swedish (e.g., Gustafsson and Nordberg, 2001) as well as along the Norwegian Skagerrak coast. The species shows significant correlations with NOI f as well as with bottom water dissolved [O2] (Fig. 3). There is no counterpart to S. fusiformis among the macrofauna as different macrofaunal species may dominate different oxygen depleted basins. In the present study, the most common macrofaunal species at the most oxygen depleted stations were the polychaetes Capitella capitata, Pseudopolydora spp. and Chaetozone setosa, and the bivalve Thyasira sarsii. This discrepancy implies a higher diversity for macrofauna compared to the foraminifera at the most stressed stations and, consequently, the present intercalibration probably gives a too high Poor/Bad class boundary for the foraminifera. However, Good/Moderate, and not Poor/Bad, is the crucial boundary within the WFD for whether or not action is needed to improve the conditions in a water body.

The successful intercalibration results justify the suggestion to accept benthic foraminifera as a Biological Quality Element within the WFD. Unique for foraminifera (as opposed to other BQEs) is their ability to quantify deviations between *in situ* reference- and present-day EcoQS based on biotic indices (for criteria, see Alve et al., 2009). Such quantifications will allow direct calculations of EQRs for the indices. Even if values of different indices may not indicate exactly the same EcoQS of present conditions, the temporal pattern revealed by different indices has been found to be very similar. In other words, the degree of change in biotic index values from the reference- to the present situation may be independent of which index is being used (Dolven et al., 2013, Fig. 6).

#### 5. Conclusions

The present study illustrates how foraminifera-based classification systems can be linked to the existing, official Norwegian monitoring system through intercalibration with macrofauna-based indices, and potentially with other NEAGIG indices.

A benthic foraminifera-based multimetric index, the Norwegian Quality Index, NQI  $_{\rm f}$ , similar to the internationally intercalibrated macrofauna-based NQI  $_{\rm m}$  is proposed.

NQI expressed a stronger relationship between foraminifera and macrofauna than the diversity indices, H'log<sub>2</sub> and ES<sub>100</sub>, but all three were acceptable for intercalibration (according to the Water Framework Directive's guidelines).

Through intercalibration, EcoQS class boundary values for the foraminifera indices were established derived from boundary values for the macrofauna. The latter were taken from the Norwegian governmental guidelines.

The study indicates that corresponding indices based on benthic foraminifera and macrofauna reflect EcoQS in a similar way. Based on the present findings and on previous studies showing the potential of fossil foraminifera to define *in situ* reference conditions and thereby to calculate EQR, it is argued that foraminifera, in addition to characterizing present day ecological status, can serve a function in biomonitoring complementary to macrofauna. It is suggested that foraminifera may be accepted as a Biological Quality Element within the WFD.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ecolind.2018.08.037.

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